Short Communication

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Decreased Exhaled Nitric Oxide in Mild Persistent Asthma Patients Treated with a Leukotriene Receptor Antagonist, Pranlukast

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Abstract: Exhaled nitric oxide (NO) level decreases after corticosteroid treatment in asthmatics, but the effect of a leukotriene receptor antagonist, pranlukast, on exhaled NO has not been elucidated. Pranlukast treatment in mild persistent asthmatics for 4 weeks decreased the exhaled NO level, which did not differ from the levels in healthy subjects. [Japanese Journal of Physiology, 49, 541–544, 1999]

Key words: nitric oxide, bronchial asthma, leukotrienes.

Nitric oxide (NO) is detected in exhaled air, and the exhaled NO level is considered to be determined by the balance between NO production in the airway (conducting airway and/or the transition zone) and NO removal into alveolar vessels [1]. The exhaled NO level is influenced by several factors, such as breath-holding time before the measurement [2], expired flow speed during the measurement [3], and cigarette smoking [4].

Another factor which influences the exhaled NO level is airway inflammation. Several studies have demonstrated significantly higher exhaled NO levels in asthma patients than in healthy subjects [5, 6]. Although it has been demonstrated that the exhaled NO level decreases after corticosteroid treatment of asthma patients [7, 8], and that glucocorticoids inhibit cytokine-induced iNOS formation in epithelial cells [9], the effect of other drugs which suppress airway inflammation and iNOS-derived NO production on exhaled NO level has not been elucidated.

Treatment with leukotriene receptor antagonists may reduce airway inflammation [10, 11], and antileukotriene therapies are becoming a potential alternative to inhaled corticosteroids for the treatment of mild persistent asthmatics [12]. An antileukotriene, pranlukast hydrate, is reported to inhibit the elevation of exhaled NO levels during the reduction of high-dose inhaled corticosteroid in moderate to severe asthma patients [13]. However, whether antileukotriene therapy without corticosteroid therapies reduces exhaled NO levels still remain unclear [14].

In this study, we examined the exhaled NO level in mild persistent asthma patients after treatment with an oral competitive leukotriene receptor antagonist, pranlukast hydrate, in an attempt to investigate the pathophysiological significance of leukotrienes in exhaled NO levels in mild persistent asthma patients. This treatment, for 4 weeks in mild persistent asthmatics without corticosteroid therapies, decreased the exhaled NO level which did not differ from the levels in healthy subjects and stable asthma patients who were treated with beclomethasone dipropionate (BDP) inhalation 200 μg twice a day.

Methods

Subjects. A total of 57 healthy non-smoker volunteers with no history of pulmonary disease and normal spirometric values served as the healthy control in this study. Twenty-two mild persistent asthma patients that were treated only with a β_2 stimulant as relief therapy were included. Each patient was carefully interviewed to determine their history, and was evalu-

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ated to confirm that they were in the asthma category of "mild persistent asthma" and that their asthma symptoms had been stable for at least 2 months. Mild persistent asthma is defined by the United States National Asthma Education Program's "Guidelines for the Diagnosis and Treatment of Asthma II" [15] as asthma that occurs on a less than daily basis, with brief attacks associated with nocturnal awakenings less than once a week but more than twice a month, and patients with normal lung function (FEV_1 or peak flow value to be more than 80% of the predicted value).

Eighteen stable asthma patients on BDP inhalation 200 μg twice a day were also included. Smokers were excluded from this study.

Pranlukast study. Twelve of the 22 mild persistent asthma patients were randomly chosen and treated with pranlukast hydrate (225 mg twice a day) for 4 weeks. As a control, the others (10 patients) were observed without pranlukast hydrate for 4 weeks.

This investigation was performed in accordance with the principles outlined in the Declaration of Helsinki, and all the subjects gave their informed consent.

Measurement of exhaled NO. After dilation of the airways by inhalation of a β_2 agoinst (salbutamol sulfate 200 μg) to minimize the effect of heterogeneous ventilation on the exhaled NO level, standing subjects expired from the maximal inspiration level after two deep breaths into a Teflon tube via a mouthpiece, and subjects were instructed to exhale against the resistance of the flow regulator to eliminate contamination by air from the nose and sinuses by closing the velum palatinum [3]. Expiration flow rate was adjusted at a constant rate of 2 *l*/min using a flow regulator. A tube with a 1.5-mm inside diameter was connected to a side port of the Teflon tube and to a chemiluminescence NO/NO_x analyzer (CLM-500, Shimadzu, Kyoto). Part of the expired air was introduced into the NO/NO_x analyzer at a constant flow rate of 50 ml/min, while the main stream was exhausted through the constant flow regulator. The exhaled NO concentration was continuously displayed on a chart recorder, and the exhaled NO level was defined as the late plateau concentration to exclude contamination by nasal air. Two measured values were averaged. To validate our method, we checked the dependency of the exhaled NO level on the expiration flow speed in healthy subjects and it was identical to the report by Silkoff *et al.* [3].

Statistical analysis. All mean values are reported with the corresponding standard deviation unless otherwise stated. The mean exhaled NO levels in the various groups were compared by ANOVA with post hoc Scheffé's test. The Friedman test was used to compare the exhaled NO levels before, 2 weeks after, and 4 weeks after the pranlukast-treatment, and the Mann-Whitney *U*-test was used to compare the exhaled NO level 4 weeks after the pranlukast-treatment and the level after 4 weeks observation of control asthma patients. p values less than 0.05 were considered significant.

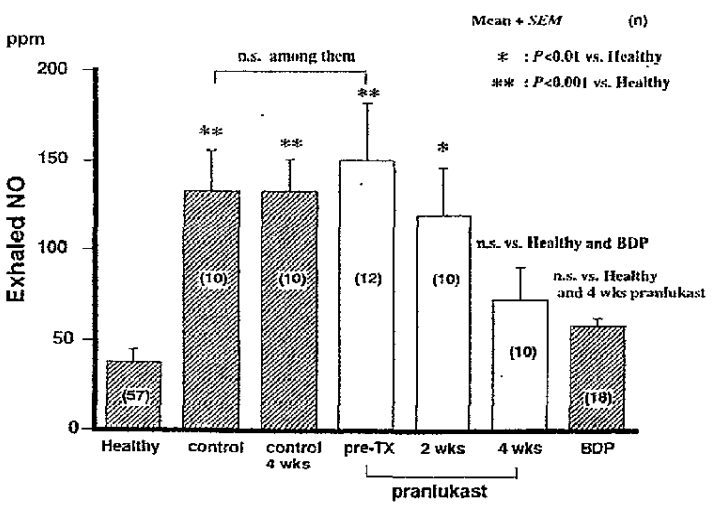

Fig. 1. Exhaled NO levels. Healthy, healthy subjects; control, mild persistent asthma patients whose exhaled NO levels were measured after 4 weeks without changing their treatment (treated only with a β_2 stimulant as relief therapy); control 4 wks, the control asthma patients after 4 weeks observation; pranlukast, mild persistent asthma patients treated with pranlukast, before the treatment (pre-TX), after treatment for 2 weeks (2 wks) and 4 weeks (4 wks); BDP, stable asthma patients who regularly inhale beclomethasone dipropionate. The exhaled NO levels of mild persistent asthma patients without administration of pranlukast hydrate were significantly higher than the level in healthy subjects, and there were no significant differences in exhaled NO values among the three groups not treated with pranlukast hydrate (control, control 4wks, and pranlukast pre-TX). After the administration of pranlukast hydrate for 2 weeks, the exhaled NO level was significantly higher than the level in healthy subjects, but after 4 weeks, the exhaled NO level did not differ from the levels in healthy subjects and stable asthma patients with BDP inhalation.

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Results

Subjects. Healthy subjects (n=57, 34 females, 23 males; age: 39.0±13.3 years; %VC, 113.3±15.7%; and %FEV_1: 104.0±14.7%), stable asthma patients on BDP inhalation therapy (n=18, 5 females, 13 males; 50.7±14.5 years; %VC, 111.2±13.0%; and %FEV_1, 96.9±14.1%), 22 mild persistent asthma patients without any treatment except β_2 stimulant inhalation as a reliever were investigated: 12 of the 22 mild persistent asthma patients were treated only with pranlukast hydrate (8 females, 4 males; age: 44.5±10.4 years; %VC, 98.1±6.1%; and %FEV_1, 87.3±9.0%), and 10 control patients (5 females, 5 males; age: 49.7±9.4 years; %VC, 99.4±20.9%; and %FEV_1, 94.6±14.5%) were observed for 4 weeks without changing the asthma treatment (β_2 stimulant inhalation as a reliever).

Exhaled NO levels. The exhaled NO levels of mild persistent asthma patients without the administration of pranlukast hydrate were significantly higher than the levels in healthy subjects (37.9±32.5 ppb, Fig. 1): control asthma patients (132.8±73.1 ppb, p=0.0009), after 4 weeks observation in control patients (132.6±73.1 ppb, p=0.0009), and before administration of pranlukast hydrate in pranlukast-treated patients (150.4±110.8 ppb, p<0.0001). There were no significant differences in exhaled NO values among the three groups not treated with pranlukast hydrate. After administration of pranlukast hydrate for 2 weeks, the exhaled NO level was 119.5±84.1 ppb, which was significantly higher than the level in healthy subjects (p=0.0046). After the administration of pranlukast hydrate for 4 weeks, the exhaled NO level further decreased to 72.9±57.3 ppb, and it did not differ from the level in healthy subjects and stable asthma patients with BDP inhalation (58.2±17.7 ppb).

In the group of asthma patients treated only with pranlukast hydrate, the exhaled NO level significantly decreased dependent on the duration of the treatment (p=0.0051, Table 1), and the exhaled NO level after 4 weeks administration of pranlukast hydrate was significantly lower than the level of the control asthma patients after 4 weeks observation without pranlukast hydrate (p=0.0376).

Table 1. Exhaled NO level in patients with mild persistent asthma with and without pranlukast.

No.	Baseline (ppb)	2 weeks (ppb)	4 weeks (ppb)
Patient (with pranlukast)			
1	285	140	n.a.
2	135	90	70
3	275	200	n.a.
4	85	n.a.	60
5	90	65	60
6	115	100	79
7	150	100	75
8	35	n.a.	10
9	30	45	30
10	115	90	65
11	95	45	55
12	395	320	225
Mean±SD	150±111	120±84	73±57
Control (without pranlukast)			
1	110		175
2	115		160
3	90		40
4	65		55
5	140		100
6	160		115
7	165		155
8	112		147
9	314		245
10	57		134
Mean±SD	132±73		133±60

n.a., not available, since these patients did not appear on the day to examine their exhaled NO level. The Friedman test revealed that the exhaled NO level significantly decreased dependent on the duration of the treatment (p=0.0051), and the exhaled NO level after 4 weeks administration of pranlukast hydrate was significantly lower than the level of the control asthma patients after 4 weeks observation without pranlukast hydrate (p=0.0376).

Discussion

Similar to the non-specific anti-inflammatory drug glucocorticoid, a 4-week administration of an oral leukotriene receptor antagonist, pranlukast hydrate, to mild persistent asthma patients also decreased exhaled NO levels to the levels of healthy subjects.

Since we did not stop BDP inhalation in the stable asthma patients who inhaled BDP, the baseline asthma status without BDP treatment in those patients could not be determined. They might be stable due to the BDP treatment, or they might be stable even without BDP treatment. However, since the exhaled NO level in the stable asthma group with BDP inhalation in this study was similar to the healthy level, BDP inhalation in mild persistent asthma patients is expected to reduce the exhaled NO level to a level equal to or slightly more than the level in healthy subjects and pranlukast-treated patients.

The 5-lipoxygenase products of arachidonic acid metabolism have been shown to be important mediators of airway inflammation and obstruction in asthma [16]. Pranlukast hydrate, a competitive leukotriene receptor antagonist, attenuates allergen-induced early and late airway responses and airway hyper-respon-

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siveness [17], and allergen-induced late asthmatic reactions have been found to be associated with the elevation of exhaled NO levels [18]. Since cysteinyl leukotrienes are produced almost exclusively by inflammatory leukocytes in humans, a blockade of the receptors may blunt TNF-α release [11], thereby reducing iNOS induction in leukocytes and bronchial epithelial cells. Furthermore, the inhibition of neutrophil recruitment will decrease the number of iNOS-positive cells. Therefore, iNOS-derived NO production would be suppressed by the blockade of leukotriene receptors.

In conclusion, a specific treatment targeted against leukotriene activity decreased the exhaled NO level in mild persistent asthma patients without glucocorticoid treatment. NO production in the airway is related to the activity of leukotrienes in mild persistent asthma.

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